

## Application of whey to prevent re-oxidation in a passive anoxic in-lake reactor – success and failure

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**Abstract** Anoxic microbial iron and sulfur reduction processes are promising tools to enhance neutralization of acidic environments. In an acidic pit lake from the lignite mine district of Lusatia, Germany (PL-111) an experimental set up (enclosure) had been installed to neutralize the sediment and the water column by adding organic substrates and lime to the sediment. Although microbial iron and sulfate reduction processes were favoured and reduced iron sulfide phases were precipitated, a substantial and progressive neutralization of the sediment and water were not achieved within 6 years after the first substrate addition. The main reason for it was assumed to be re-oxidation of the reduced mineral phases, probably by oxygen supply during seasonal overturn of the water column. In October 2007, a cover plane was mounted on top of the enclosure to protect it against oxygen input. Regular supply of whey powder to the water was done over one year to provide stable anoxic conditions within the enclosure. To control the success of this measure, bi-monthly sampling of the water column was conducted including physico-chemical parameters. Two intense sampling campaigns of the sediment and pore-water were performed just before starting and one year after the first addition of whey powder. Repeated whey additions kept the bottom water anoxic, but did not stimulate Fe(III) reduction in the water column. Iron sulfide phases in the sediment had not increased one year after enclosure coverage and whey addition, although microbial sulfate reduction proceeded in the sediment. This implies that re-oxidation of sulfides could not be prevented by the tested measures.

**Key Words** acidic pit lake, iron reduction, sulfate reduction, sediment chemistry

### Introduction

Neutralization of acidic mine waters may be achieved by reductive microbial processes as for example by iron and sulfate reduction if stable iron and metal sulfide precipitates are formed (e.g. Gazea et al. 1996, Younger et al. 2002). This concept which is generally used within constructed wetlands (e.g. Hedin et al. 1994, Younger et al. 2002) was also tested for acidic pit lakes from lignite mining in Germany (e.g. Klapper et al. 1998, Wendt-Potthoff et al. 2002; 2010; Frömmichen et al. 2003, 2004; Koschorreck et al. 2007; Geller et al. 2009). In the case of acidic pit lakes which generally are poor in nutrients (low phosphorus, low carbon), the application of organic substrates as electron donors to enhance heterotrophic bacterial processes is a prerequisite. Furthermore, the reductive processes require anoxic conditions which can be found in the sediments if meromictic conditions or at least anoxic hypolimnia occur. In other cases where the acidic pit lakes exhibit dimictic or polymictic situations throughout a year anoxic conditions have to be maintained by biotechnological measures. Otherwise, re-oxidation of the iron and metal sulfides by oxygen will occur at the sediment surface. Prevention of oxygen mixing to the lake bottom includes also prevention of algae growth which produce oxygen during photosynthesis. This conflicts with the supply of organic substrates which favors the growth of algae and other phytoplankton in lakes. Beside oxygen, ferric iron is a further oxidant which is normally produced by oxidation of ferrous iron entering the pit lakes from ground- and dump waters. Therefore, it was the task to find a method which could increase bacterial reductive processes, establish anoxic conditions at the lake bottom and minimize oxygen mixing and algae growth.

### Study site and methods

Experiments to neutralize an acidic pit lake were carried out from 2001 to 2006 by applying straw and "Carbokalk" (a by-product of the sugar industry) to a field-enclosure of 30 m in diameter and 6.5 m in depth installed in pit lake PL-111 (Wendt-Potthoff et al. 2002; Frömmichen et al. 2003, 2004; Bozau et al. 2007; Koschorreck et al. 2007; Geller et al. 2009). Pit Lake 111 of the Lusatian lignite mining district (Germany) has a surface area of about 10.7 ha, a volume of approx.  $0.5 \cdot 10^6 \text{ m}^3$ , a mean depth of 4.6 m and a maximum depth of 10 m (Büttner et al. 1998). The lake has a pH of

2.6, and a Ca-SO<sub>4</sub> chemistry with sulfate concentrations of about 1200 mg/L, iron concentrations around 150 mg/L and Al concentrations of about 40 mg/L (Herzsprung et al. 1998).

Unfortunately, only low rates of net gain in alkalinity were observed over the duration of the experiments within the sediment of the field enclosure. The main reason for this failure was assumed to be re-oxidation of the iron and metals sulphides by bottom mixing of oxygen during spring and autumn overturn. Therefore, an application of whey to the water column of the field enclosure was performed in September 2007 to reduce all molecular oxygen and establish anoxic conditions. Additionally, the field enclosure was covered by a floating foil to reduce oxygen input and to prevent light penetration for inhibiting algae growth.

To choose whey as a reactant for reducing oxygen was the result of a competitive batch experiment with glucose, ethanol, methanol, acetate, and lactate among others (Wendt-Potthoff et al. 2008). The amount of whey which has to be applied to reduce all the oxygen was calculated on electron balance. Taking a vertical oxygen profile measured in the water column and the volume of the field enclosure of 4500 m<sup>3</sup> into account an oxygen content of about 1260 moles O<sub>2</sub> equivalent to 5040 moles of electrons was calculated. From the batch experiments it was known that 1 kg of whey was consuming 120 mol electrons of O<sub>2</sub>. Therefore, theoretically 42 kg of whey should be sufficient to respire all dissolved oxygen from the water column. On October, 9<sup>th</sup> 2007, 50 kg of whey were supplied to the field enclosure and the enclosure was covered subsequently by the floating foil. Because the monitoring of the oxygen concentration during the experiment showed a decrease in O<sub>2</sub>-respiration after three weeks (see below) additional whey application of 50 kg each were conducted in November 2007, April 2008, and August 2008.

Oxygen concentrations were monitored in weekly to monthly intervals of vertical O<sub>2</sub>-profiling with a multiparameter probe (Idronaut, Italy), by using a hand-held oxygen meter (WTW, Germany) and for the start-up phase additionally by an optical oxygen sensor (Presens, Germany) installed at 2 m water depth, recording the O<sub>2</sub> concentration hourly. Just before applying the whey undisturbed sediment cores of 20 cm length were retrieved by gravity corer technique and separated cm by cm for the top 5 cm within a glove box to maintain anoxic conditions during preparation. The same sampling protocol was performed after one year of experiment run in October 2008 to compare the effects within the sediment before and after whey addition. From the top 5 cm in each cm of the sediment cores pH and Eh were measured by punching in pH and ORP electrodes (WTW, Germany). Eh was corrected for temperature to standard hydrogen electrode at pH 7 (EH<sub>7</sub>). As a measure for the extent of reducing processes the total amount of reduced inorganic sulfur (TRIS) was used. Thereby, TRIS was calculated as the sum of acid volatile sulfur (AVS), chromium reducible sulfur (CRS), and elemental sulfur (ES) which were extracted sequentially in a modified three-step method according to Frömmichen et al. (2001) after the methods of Canfield (1989), Fossing and Jørgensen (1989), and Hsieh and Yang (1989). The extracted hydrogen sulfide was trapped in a sulfur antioxidant buffer and was measured with differential pulse polarography.

## Results and Discussion

The application and distribution of whey within the enclosure was unproblematic from a technical point of view. The whey was completely dissolved and detected as dissolved organic carbon (DOC) in the water. Immediately after the addition of whey, respiration of oxygen started and the rate of oxygen consumption increased steadily for about 3 weeks. Thereafter, the rate slowed down to a constant rate of 4.3 μmol L<sup>-1</sup> d<sup>-1</sup>. Additional supply of whey increased the respiration rate of oxygen to a maximum rate of 42 μmol L<sup>-1</sup> d<sup>-1</sup>. One month after whey addition the enclosure was completely anoxic below 1 m water depth.

The pH of the sediment before whey addition increased from around 3 at the surface to 6 within the top 5 cm of sediment depth (Table 1). In contrast, the pH distribution in the sediment of the lake outside of the field enclosure remained at acid values of 2.7 down to 15 cm. The remarkably higher pH values in the sediment from the field enclosure were probably the effect of 5 years of experimental neutralizing with addition of straw and Carbokalk. The pH profiles were similar to previous studies in the enclosure (Koschorreck et al. 2007). In 2008, the pH of the enclosure sediment showed nearly identical values as in 2007 (Tab. 1). Eh values are only available for 2008 since the electrode was broken in 2007 during sampling. The redox potential was decreasing with sediment depth but the absolute values were not extremely low (e.g. above -200 mV). Finally, the amount of TRIS was nearly identical between 2007 and 2008 showing the same depth distribution

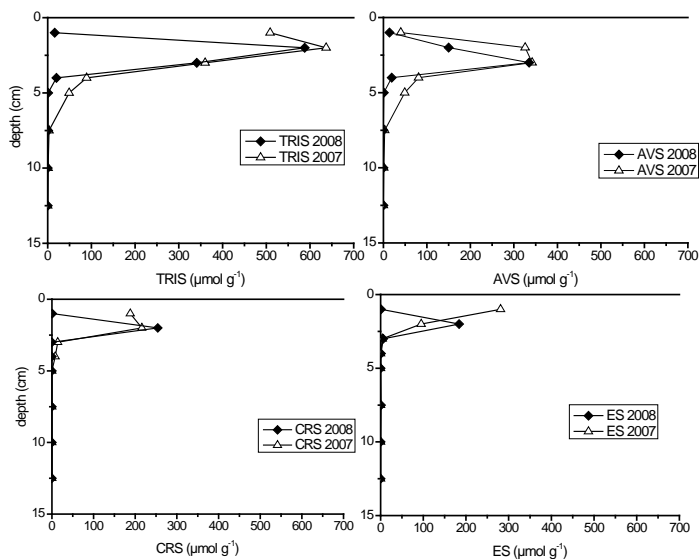
**Table 1** pH and  $E_{H7}$  values in the top 5 cm of sediment cores from the field enclosure from 2007 and 2008 in comparison with the untreated sediment from pit lake 111 ( $E_{H7}$  are temperature corrected Eh values to the standard hydrogen electrode at pH 7)

Sediment depth (cm)	pH						$E_{H7}$ (mV)	
	lake	2007 enclosure		2008 enclosure		lake	2008 enclosure	
		core 1	core 2	core 3	core 4		core 3	core 4
-0.5	2.6	3.6	3.2	3.1	5.3	682	377	137
-1.5	2.6	4.8	4.0	4.2	5.4	653	167	67
-2.5	2.6	6.0	5.3	4.8	5.8	634	117	-93
-3.5	2.6	6.1	5.6	5.4	5.9	622	37	-23
-4.5	2.7	6.2	5.9	5.9	6.0	609	-3	-23

(Figure 1). About two thirds of the TRIS consisted of the more labile AVS, the rest were comparable parts of the relatively crystalline CRS and ES. This speciation of TRIS did not change significantly during the experiment. Thus, there was no trend towards more stable sulfide minerals during the experiment.

**Conclusions**

Although it was possible to reduce the oxygen within the field enclosure within one month and maintain the anoxic conditions for at least one year, there was no increase of TRIS accumulation observed in the sediment and the stability of the sulfide minerals was not increased. Consequently, pH values remain constant over the year and no neutralizing effect was possible for the water column. The experiment demonstrated that the solely application of whey is not sufficient to increase the net neutralisation rate in passive mine lake treatment.



**Figure 1** Comparison of the depth distribution of TRIS (total reduced inorganic sulfur) AVS (acid volatile sulfur), CRS (chromium reducible sulfur) and ES (elemental sulfur) on dry weight basis ( $\mu\text{mol/g}$ ) in sediment cores from the field enclosure for 2007 (open symbols) and 2008 (closed symbols)

## Acknowledgements

The authors thank Kerstin Lerche, Corinna Völkner, Michael Herzog, Burkhard Kuehn, Karsten Rahn, and Martin Wieprecht for assistance in the field and lab.

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